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International Bureau

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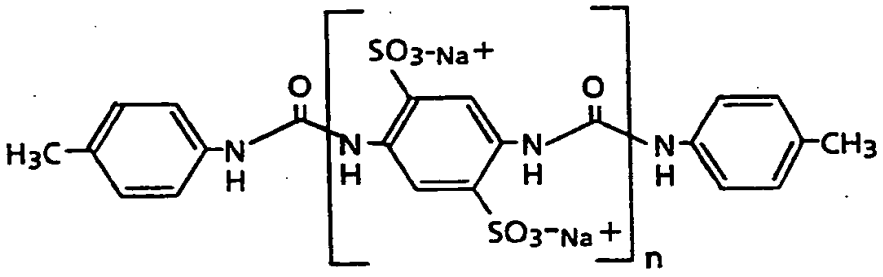
Published
With international search report.
With amended claims.

(54) Title: METHOD FOR ANTAGONIZING INOSITOL 1,4,5-TRIPHOSPHATE



(I)

(57) Abstract

Oligomers of formula (I) have been demonstrated to be effective antagonists of inositol 1,4,5-triphosphate (IP_3) by competitively vying with IP_3 for binding sites. By competitively inhibiting the activity of IP_3, the oligomers of this invention can modulate the release of intracellular calcium and elicit the resultant physiological effects.

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METHOD FOR ANTAGONIZING INOSITOL 1,4,5-TRISPHOSPHATE

BACKGROUND OF THE INVENTION

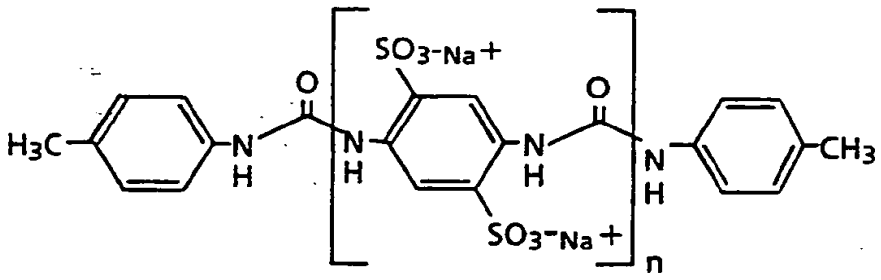
This application relates to a series of diamino benzenedisulfonic acid oligomers that have demonstrated an affinity for the receptor sites of inositol 1,4,5-triphosphate (IP_3) and are, therefore useful in diminishing the bioactivity of IP_3, especially with regard to its effect on the release of intracellular calcium ions.

DESCRIPTION OF THE PRIOR ART

The diamino benzenedisulfonic acid oligomers demonstrating utility as IP_3 antagonists according to this invention are described in detail in the European Patent Application published January 22, 1992 under Publication No. 0467185 A2. In that publication, the oligomers of the present invention were described as having utility in the diagnosis and/or treatment of AIDS and AIDS related complex.

SUMMARY OF THE INVENTION

The invention herein disclosed relates to a method of inhibiting the activity of inositol 1,4,5-triphosphate (IP_3) by occupying the receptor sites specific to IP_3 with a compound of the formula:



wherein n is a whole number selected from the range of 5 to 20 inclusive and the pharmaceutically acceptable salts thereof.

DETAILED DESCRIPTION OF THE INVENTION

Inositol 1,4,5-triphosphate (IP_3) is a naturally occurring and active component of animal physiology. It is formed intracellularly upon the activation of cell-surface receptors linked to the enzyme phospholipase C. Once generated in sufficient quantities, IP_3 acts to stimulate the release of calcium ions from storage organelles within the cell. In this role IP_3 is characterized as a "second messenger". Depending upon the type of cell, the calcium released by IP_3 functions to stimulate a variety of physiologic processes such as smooth muscle contraction, histamine secretion and the hyperpolarization of nerve cells. Any compound or agent that can promote or interfere with the function of IP_3, will promote or interfere with the generation of calcium ions and thereby elicit predictable pharmacological effects.

The process by which IP_3 releases calcium ions begins with the binding of IP_3 to a specific receptor protein located on an intracellular calcium storage compartment located typically on the endoplasmic reticulum. This receptor protein has been cloned and has been shown to form a calcium "channel" with unique structural properties when bound to IP_3. Therefore, when IP_3 binds with its receptor, a calcium channel is opened causing the release of calcium stored in the cell's endoplasmic reticulum. In turn, the

released calcium will elicit the appropriate cellular response.

Heretofore, the only verified potent antagonist of the IP_3 receptor was heparin, a complex glycosaminoglycan. The diamino benzenedisulfonic acid oligomers of this invention also appear to antagonize the effects of IP_3 by competing for the receptor site. In most cases, these compounds are more effective than heparin and demonstrate fewer secondary effects. In addition to providing utility as laboratory "tools" in evaluating the therapeutic potential of other IP_3 receptor antagonists, the oligomers of this invention would also be administered to modulate IP_3-induced calcium release and have a salutary effect on any number of disorders that are caused or exacerbated by an inordinately productive IP_3 second messenger pathway.

EXPERIMENTALS

Measurement of IP_3 Binding

Cerebella from male Sprague-Dawley rats (200 g) were homogenized in 30 volumes of ice-cold buffer A (50 mM Tris, 1 mM dithiothreitol, 1 mM EDTA, pH 7.7 with HCl) with a polytron (setting 9 for 10 seconds). The tissue is then washed twice by centrifugation (20,000 x g, 15 minutes; Sorvall 28-S., SS-34 rotor) and resuspended in 30 volumes of ice-cold buffer A.

For the binding assays, 1.5 ml eppendorf tubes containing 50 µl of test compound (made up as a 10x stock in water) or water, 50 µl [3H] IP_3 (17Ci/mmol; Dupont-NEN; usually made as a 25 nM (10x) stock solution in buffer), and 350 µl of buffer B (buffer A with pH adjusted to 8.4) on ice. Tubes for non-specific binding also contained 50 µl of non-radioactive IP_3 (100 µM stock (10x); final concentration 10 µM), with an appropriate reduction in the volume of buffer B. Reactions were initiated by the addition of 50 µl tissue to make the final volume 500 µl,

followed by vortex mixing. Samples were incubated on ice for 10 minutes and then were centrifuged (14,000 x g) in a microfuge (Eppendorf model 5415) for 5 minutes followed by aspiration of the supernatant fraction. The tissue pellets were solubilized overnight in 100 µl of Protosol (Dupont-NEN). After solubilization, 73 µl of glacial acetic acid were added to decrease chemiluminescence, and the mixture was transferred to scintillation vials. To these vials was added 7 ml of Ecoscint-A (National Diagnostics) and the radioactivity determined by liquid scintillation spectrophotometry.

Specific binding was defined as the difference between total binding (radioactivity in the absence of test compound and cold IP_3) and non-specific binding (radioactivity in the absence of test compound but in the presence of cold IP_3). This number was taken as 100% specific binding. Data points obtained with the test compounds were fit by a computer program (GraphPad-InPlot) to determine their inhibitory potency. The inhibitory potencies of the test compounds were expressed as the concentration of compound that produces 50% inhibition of specific binding (the IC_{50} value).

The binding data are presented in Table 1 and demonstrate that compounds within the scope of the present invention effectively compete for [3H] IP_3 binding sites in rat cerebellar membranes. The compound identified as MDL 102,869 was the most potent competitor for binding with an IC_{50} of 50 nM, whereas low molecular weight heparin (5100 MW) had an IC_{50} of 74 nM. MDL 102,869 is the compound according to the claimed invention wherein n = 15.

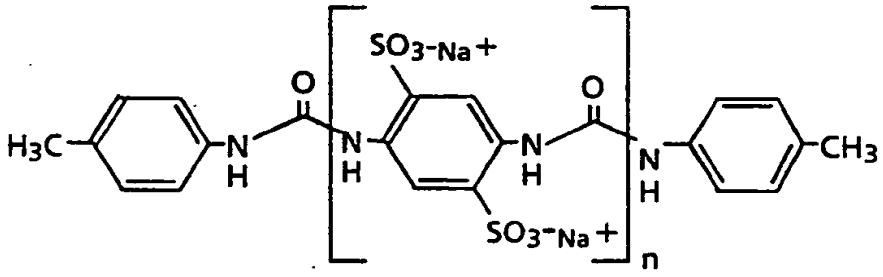
The potency for binding also seems to correlate with the ability to antagonize IP_3-induced calcium ion release. Thus, 1 and 3 µM of MDL 102,869 inhibited calcium ion release by 42 and 100%, respectively (see Fig. 1), whereas

10 μM of heparin inhibited release by 90%. MDL 101,828, which had an IC_{50} binding of 104 nM, inhibited IP_3-induced calcium ion release by 72% at 3 micro moles. MDL 101,828 is the compound according to the claimed invention wherein n = 9.

TABLE 1

Compound	Binding IC_{50} (nM)	Inhibition of $Ins(1,4,5)P_3$-Induced Ca^{2+}-release
Heparin (low MW)	74	90% at 10 μM
MDL 101,828	104	42% at 3 μM
MDL 102,869	50	42% at 1 μM 100% at 3 μM

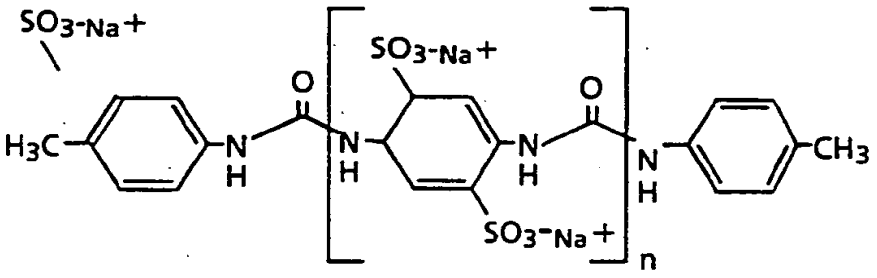
The tracing of Fig. 1, dramatically illustrates the IP_3 inhibition data set forth in the third column of Table 1.



The y-axis represents the concentration of free calcium ions in arbitrary units. The tracing shows that two successive additions of 0.1 μM of IP_3 stimulated similar amounts of calcium ion release from cerebellar microsomes. The addition of 1 μM of MDL 102,869 stimulated a small increase of calcium ion for unknown reasons. In the presence of 102,869, however, calcium ion release stimulated by 0.1 μM of IP_3 was inhibited by 42%. This inhibition was overcome by the addition of 1 μM of IP_3, consistent with competitive antagonism by MDL 102,869.

WHAT IS CLAIMED IS:

1. A method of inhibiting the activity of inositol 1,4,5-trisphosphate by occupying a receptor site specific to inositol 1,4,5-trisphosphate with a compound of the formula:



wherein n is a whole number within the range of 5-20 and the pharmaceutically acceptable salts thereof.

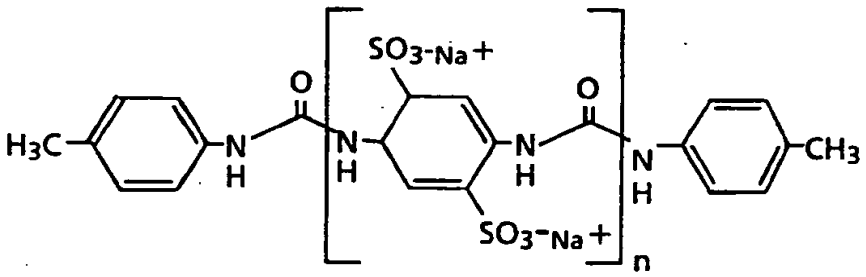
2. The oligomer of claim 1 wherein n is 9.

3. The oligomer of claim 1 wherein n is 15.

AMENDED CLAIMS

[received by the International Bureau on 11 January 1994 (11.01.94); original claims 2-3 replaced by amended claims 2-3; other claims unchanged (1 page)]

1. A method of inhibiting the activity of inositol 1,4,5-trisphosphate by occupying a receptor site specific to inositol 1,4,5-trisphosphate with a compound of the formula:



wherein n is a whole number within the range of 5-20 and the pharmaceutically acceptable salts thereof.

2. The method of claim 1 wherein n is 9.

3. The method of claim 1 wherein n is 15.

INTERNATIONAL SEARCH REPORT

Inter. ional Application No
PCT/US 93/08168

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K31/795

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 467 185 (THE DOW CHEMICAL COMPANY) 22 January 1992 cited in the application	1
X	see abstract; claims; example 3 ---	2,3
Y	THE BIOCHEMICAL JOURNAL vol. 267, no. 2 , 1990 pages 297 - 302 F. O'ROURKE ET AL. 'THE INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR BINDING SITES OF PLATELET MEMBRANES' see the whole document ---	1
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
14 December 1993	23. 12. 93

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Hoff, P

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter. ...nal Application No
PCT/US 93/08168

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FEBS LETTERS vol. 252, no. 1,2 , 1989 pages 105 - 108 M.A. TONES ET AL. 'THE EFFECT OF HEPARIN ON THE INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR IN RAT LIVER MICROSOMES' see the whole document	1
A	THE JOURNAL OF BIOLOGICAL CHEMISTRY vol. 263, no. 23 , 1988 pages 11075 - 11079 T.K. GHOSH ET AL. 'COMPETITIVE, REVERSIBLE, AND POTENT ANTAGONISM OF INOSITOL 1,4,5-TRISPHOSPHATE-ACTIVATED CALCIUM RELEASE BY HEPARIN' see the whole document	1
A	GB,A,781 479 (CIBA LIMITED) 21 August 1957 see page 2, line 8 - line 30; claims 1,2,9-12,22; example 5	1-3

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. ..ional application No.
PCT/US93/08168

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 1 is directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of th e compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. nal Application No
PCT/US 93/08168

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0467185	22-01-92	AU-B-	635850	01-04-93
		AU-A-	8024291	09-01-92
		AU-A-	8286791	04-02-92
		CA-A-	2046491	10-01-92
		CN-A-	1058959	26-02-92
		EP-A-	0538373	28-04-93
		JP-A-	4226521	17-08-92
		WO-A-	9200749	23-01-92
GB-A-781479		NONE		

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